

## We claim:

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- An isolated polynucleotide derived from a fungal source, which polynucleotide comprises a nucleotide sequence encoding an enzyme having β-glucosidase activity.
- 5 2. An isolated polynucleotide selected from the group consisting of:
  - (a) a nucleic acid sequence which encodes or is complementary to a sequence which encodes a BGL4 polypeptide having at least 85% sequence identity to the amino acid sequence presented in Figure 2 (SEQ ID NO:2);
  - (b) a nucleic acid sequence which encodes or is complementary to a sequence which encodes a BGL4 polypeptide having at least 90% sequence identity to the amino acid sequence presented in Figure 2 (SEQ ID NO:2);
  - (c) a nucleic acid sequence which encodes or is complementary to a sequence which encodes a BGL4 polypeptide having at least 95% sequence identity to the amino acid sequence presented in Figure 2;
  - (d) a nucleic acid sequence which encodes or is complementary to a sequence which encodes a BGL4 polypeptide having the amino acid sequence presented in Figure 2;
  - (e) a nucleic acid sequence which encodes or is complementary to a sequence which encodes a BGL4 polypeptide having at least 95% sequence identity to the amino acid sequence presented as SEQ ID NO:2;
  - (f) a nucleic acid sequence which encodes or is complementary to a sequence which encodes a BGL4 polypeptide having the amino acid sequence presented as SEQ ID NO:2;
  - (g) a nucleic acid sequence presented as SEQ ID NO:3, or the complement thereof; and
  - (h) a nucleic acid sequence that hybridizes, under high stringency conditions to the sequence presented as SEQ ID NO:3, or the complement or a fragment thereof, wherein said isolated polynucleotide encodes a polypeptide having the biological activity of a β-glucosidase
  - 3. The isolated polynucleotide of Claim 2, wherein % identity is calculated using the CLUSTAL-W program in MacVector version 6.5, operated with default parameters, including an open gap penalty of 10.0, an extended gap penalty of 0.1, and a BLOSUM 30 similarity matrix.

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- 4. The isolated polynucleotide of Claim 2, wherein hybridization is conducted at 42°C in 50% formamide, 6X SSC, 5X Denhardt's solution, 0.5% SDS and 100 μg/ml denatured carrier DNA followed by washing two times in 2X SSPE and 0.5% SDS at room temperature and two additional times in 0.1 SSPE and 0.5% SDS at 42°C.
- 5. The isolated polynucleotide of Claim 2, wherein said polynucleotide is an RNA molecule.
- 6. The isolated polynucleotide encoding an enzyme having  $\beta$ -glucosidase activity, wherein the enzyme is derived from a Trichoderma source.
- 7. The isolated polynucleotide of Claim 6, wherein the enzyme is derived from Trichoderma reesei.
  - 8. An expression construct including a polynucleotide sequence (i) having at least 85% sequence identity to the amino acid sequence presented in Figure 2 (SEQ ID NO:2), or (ii) being capable of hybridizing to a probe derived from the nucleotide sequence disclosed in Figure 2 under conditions of intermediate to high stringency, or (iii) being complementary to a nucleotide sequence having at least 85% sequence identity to the amino acid sequence presented in Figure 2 (SEQ ID NO:2).
  - 9. A vector including the expression construct of Claim 8.
  - 10. A vector comprising an isolated polynucleotide of Claim 2, operably linked to control sequences recognized by a host cell transformed with the vector.
  - 11. A host cell transformed with the vector of Claim 9.,
  - 12. A host cell transformed with the vector of Claim 10.
  - 13. The host cell of Claim 12, which is a prokaryotic cell.
  - 14. The host cell of Claim 12, which is a eukaryotic cell.
    - 15. A recombinant host cell comprising a polynucleotide of Claim 2.
    - 16. The recombinant host cell of Claim 15, which is a prokaryotic cell.
    - 17. The recombinant host cell of Claim 15, which is a eukaryotic cell.
    - 18 A substantially purified BGL4 polypeptide with the biological activity of a  $\beta$ -gluogsidase, comprising a sequence selected from the group consisting of:
      - (a) an amino acid sequence having at least 85% sequence identity to the amino acid sequence presented in Figure 2 (SEQ ID NO:2);
      - (b) an amino acid sequence having at least 90% sequence identity to the amino acid sequence presented in Figure 2 (SEQ ID NO:2);
      - (c) an amino acid sequence having at least 95% sequence identity to the amino acid sequence presented in Figure 2;
      - (d) an amino acid sequence presented in Figure 2;

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PATENT
Attorney Docket No. GC696

(e) an amino acid sequence having at least 95% sequence identity to the amino acid sequence presented as SEQ ID NO:2;

- (f) an amino acid sequence presented as SEQ ID NO:2;
- (g) a substantially purified biologically active fragment of the amino acid sequence presented as SEQ ID NO:2.
- 19. A method of producing an enzyme having  $\beta$ -glucosidase activity, comprising:
  - (a) stably transforming a host cell with an expression vector comprising a polynucleotide as defined in Claim2;
  - (b) cultivating said transformed host cell under condition suitable for said host cell to produce said β-glucosidase; and
  - (c) recovering said  $\beta$ -glucosidase.
- 20. The method of Claim 19 wherein the host cell is a filamentous fungi or yeast cell.
- 21. A purified enzyme having  $\beta$ -glucosidase activity prepared by the method of Claim 19...
- 22. A recombinant host cell comprising a deletion or insertion or other alteration in the bgl4 gene which inactivates the gene and prevents BGL4 polypeptide production.
  - 23. An antisense oligonucleotide complementary to a messenger RNA that encodes a BGL4 polypeptide having the sequence presented as SEQ ID NO:2, wherein upon exposure to a  $\beta$ -glucosidase-producing host cell, said oligonucleotide decreases or inhibits the production of  $\beta$ -glucosidase by said host cell.
  - 24. The antisense oligonucleotide of Claim 23, wherein the host cell is a filamentous fungi.
  - 25. A detergent composition, said composition comprising a polypeptide selected from the group consisting of:
    - (a) an amino acid sequence having at least 85% sequence identity to the amino acid sequence presented in Figure 2 (SEQ ID NO:2);
    - (b) an amino acid sequence having at least 90% sequence identity to the amino acid sequence presented in Figure 2 (SEQ ID NO:2);
    - (c) an amino acid sequence having at least 95% sequence identity to the amino acid sequence presented in Figure 2;
    - (d) an amino acid sequence presented in Figure 2;
    - (e) an amino acid sequence having at least 95% sequence identity to the amino acid sequence presented as SEQ ID NO:2;
    - (f) an amino acid sequence presented as SEQ ID NO:2;
    - (g) a substantially purified biologically active fragment of the amino acid sequence presented as SEQ ID NO:2.

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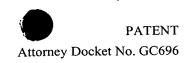
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- 26. A method of expressing a heterologous polypeptide having  $\beta$ -glucosidase activity in an Aspergillus species, comprising:
  - (a) Providing a host Aspergillus with an expression vector comprising a
    polynucleotide encoding a signal sequence linked to a polynucleotide
    encoding a heterologous β-glucosidase, thereby encoding a chimeric
    polypeptide;
  - (b) Cultivating said host Aspergillus under conditions suitable for said Aspergillus to produce said chimeric polypeptide, wherein said chimeric polypeptide is produced.
- 27. A method of producing ethanol, said method comprising the steps of:
  - contacting a biomass composition with an enzymatic composition comprising β-glucosidase 4 to yield a sugar solution;
  - b) adding to the sugar solution a fermentative microorganism; and
  - c) culturing the fermentative microorganism under conditions sufficient to produce ethanol,

wherein the biomass composition may be optionally pretreated.

- 28. The method of claim 27 wherein step (a) further comprises the addition of at least one endoglucanase.
- 29. The method of claim 27 wherein step (a) further comprises the addition of at least one cellbiohydrolase.
- 30. The method of claim 28 wherein step (a) further comprises the addition of at least one cellbiohydrolase.
- 31. The method of claim 27 wherein the pretreatment is with a dilute acid.
- 32. A method of producing ethanol, said method comprising the steps of:
  - a) contacting a biomass composition with an enzymatic composition comprising a  $\beta$ -glucosidase 4 and a fermentative microorganism; and
  - culturing the fermentative microorganism under conditions sufficient to produce ethanol,

wherein the biomass composition may be optionally pretreated.

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  33. The method of claim 32 wherein step (a) further comprises the addition of at least one endoglucanase.
- 34. The method of claim 32 wherein step (a) further comprises the addition of at least one cellbiohydrolase.
  - 35. The method of claim 33 wherein step (a) further comprises the addition of at least one cellbiohydrolase.
- 36. The method of claim 32 wherein the pretreatment is with a dilute acid.